Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds

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ABSTRACT: A reduction in food intake is a prominent feature of many infectious diseases. However, the underlying mechanisms of parasite-induced anorexia in sheep are poorly understood. Here, we tested the hypotheses (a) that the degree of parasite-induced anorexia in lambs is influenced by their growth potential and (b) that nematode infection results in elevated plasma leptin concentration in lambs. The hypotheses were tested with Suffolk × Greyface (S) and Scottish Blackface (B) lambs that are known to differ in their growth potential (S lambs are of greater growth potential than B lambs). During a primary parasite infection, 24 out of 48 lambs per breed were trickle-infected with 7,000 infective Teladorsagia circumcincta larvae per day, 3 d/wk, for a period of 12 wk (experiment I). The lambs were then dewormed, and after a 2-wk interval, half of the 24 lambs per breed that were previously infected were reinfected for another 12 wk with the same parasite and dose as used in the primary infection (experiment II). In both experiments, infected lambs were fed grass pellets for ad libitum intake, whereas noninfected lambs were fed grass pellets for either ad libitum or restricted intakes. The S lambs were more susceptible than B lambs to nematode infection, as judged from the differences in fecal egg counts ($P = 0.007$). Parasitized lambs of the more susceptible breed (S) showed anorexia [i.e., a decrease in intake of 13% compared with uninfected controls ($P = 0.01$)], whereas no significant reduction in food intake was observed in lambs of the more resistant breed (B). Reexposure to nematode infection of previously infected animals tended to result in renewed anorexia in S lambs but not in B lambs ($P = 0.08$) in a similar extent as during primary infection. Plasma leptin concentrations did not differ between ad libitum-fed infected and control lambs but were greater in infected than in noninfected lambs at a similar level of food intake during both the primary ($P = 0.02$) and the secondary parasitic infection ($P = 0.004$) in both breeds. The results show that leptin may be involved in the response of lambs to infection but that it is unlikely that leptin alone is responsible for the parasite-induced anorexia in lambs.

Key words: anorexia, breed, infection, leptin, nematode, sheep

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INTRODUCTION

Anorexia associated with gastrointestinal nematode infections has a significant effect on productivity in livestock grazing systems. However, the underlying mechanisms that induce and maintain anorexia in infected sheep remain unclear. The recent literature suggests that anorexia after nematode infection in lambs is likely to be related to the development of the immune response (Greer et al., 2005). Recent evidence from studies in rodents indicates that the adipocyte hormone leptin plays an important role in the regulation of immune responses. Plasma leptin concentrations (PLC) and ADFI are related (Henry et al., 1999), and PLC increase during infection and inflammation in many models of disease (Faggioni et al., 2001). Whether PLC...
increase during nematode infections in sheep, and if such increases in leptin are associated with parasite-induced anorexia, is not known.

Sandberg et al. (2006) postulated in a recently developed model that a genotype more susceptible to parasitic infection, as assessed by fecal egg count (FEC), is likely to exhibit a greater degree of anorexia compared with a resistant genotype. Host immune responses to infection can be compromised by the needs for maintenance and growth (Coop and Kyriazakis, 1999), and therefore, differences in resistance to nematode infection can be expected between breeds that differ in their intrinsic capacity for growth. However, whether breeds of sheep that differ in their growth potential differ also in the degree of anorexia after infection is not known.

In the current study, we test the hypotheses that a) nematode infection will result in a greater extent and longer duration of anorexia in lambs of high growth potential than in lambs of lower growth potential and b) infected lambs will have elevated PLC compared with noninfected lambs at a similar level of food intake. Both hypotheses were tested during a primary and a secondary trickle infection in 2 consecutive experiments.

MATERIALS AND METHODS

The experiments took place at the facilities of the Scottish Agricultural College after approval of the experimental protocol by the Animal Experiments Committee and under Home Office license for experimental infection and blood sampling.

Experiment I (Primary Infection)

Animals, Husbandry Procedures, and Housing. Ninety-six weaned lambs, 48 Suffolk × Greyface crosses (S) and 48 Scottish Blackface (B) were used. The lambs were approximately 12 wk of age, and half of them were male and half female within each breed. All lambs were born indoors and, soon after birth, were transferred along with their mothers (as a single flock) onto a pasture that was newly plowed and seeded and had never been grazed before in an attempt to maintain it parasite-free. Before lambing, the ewes had been orally drenched with a combination of fenbendazole (Panacur 10%, Hoechst Roussel Vet Ltd., Milton Keynes, Buckinghamshire, UK) at a rate of 0.2 mL/kg of BW and levamisole (Nilverm Gold, Schering-Plough, Welwyn Garden City, UK) at a rate of 7.5 mg/kg of BW, to remove worm burdens and ensure that lambs would not become infected from contact with the ewe-derived parasites deposited onto the pasture. The lambs remained on this pasture until weaning at around 10 wk of age. After weaning, the lambs were brought into a naturally illuminated and ventilated shed and housed in individual pens, measuring 2.0 × 1.5 m, until the end of the experiment. All pens contained a food trough that allowed measurement of individual ADFI and a water bowl that gave animals continuous access to water. Fresh food was supplied in 2 daily portions (early morning and late afternoon). Lambs were acclimatized for a period of 3 wk before the beginning of experimental observations. At the end of this period, S and B lambs had mean initial BW of 30.1 ± 0.32 kg and 21.3 ± 0.43 kg, respectively. Four additional sheep were housed separately and used as larvae donors during the experiments.

Experimental Design. Lambs were assigned randomly to treatments on the basis of their breed, sex, and initial BW, ensuring an equal number of males and females of similar BW within both breeds on each treatment. To ensure the availability of sufficient animals for the reinfection experiment (see below), half of all lambs (24 of each breed) were assigned to the infection treatments. Of the remaining control lambs, half (12 of each breed) were fed for ad libitum intake to allow estimation of anorexia in infected lambs. To measure the effects of ADFI on PLC, the remaining control lambs were assigned to 1 of 3 restricted feeding levels (4 lambs of each breed per feeding level).

All lambs in the infection treatment (INF) were dosed with 7,000 infective third-stage Teladorsagia circumcincta larvae (L3) every Monday, Wednesday, and Friday. The trickle infection began on d 0 and ceased after 12 wk (d 84). Each infective dose was suspended in 10 mL of water and was administered orally using a syringe. The L3 originated from an anthelmintic-susceptible strain that had been donated by the Moredun Research Institute (Edinburgh, UK). Larvae were harvested every 14 d from feces of a monospecifically infected donor sheep using a standard Baermann procedure. After harvesting, L3 were stored at 4°C in tap water (700 L3/mL) and used within 3 wk of collection. All control lambs were given a similar volume of water (sham infection) at the same time, thus undergoing the same amount of handling stress as the infected animals. On d 84, INF lambs were drenched with a combination of fenbendazole and levamisole (same dose as above) to remove worm burdens. These lambs remained in the shed and were used in experiment II, whereas noninfected lambs were returned to stock.

Lambs received grass pellets with an average composition (per kg as fed) of 935 g of DM, 186 g of CP, 516 g of NDF, and 9.5 MJ of ME according to the feed supplier analyses. Infected lambs were fed for ad libitum intake throughout the experiment, whereas noninfected lambs were fed either for ad libitum intake (Calis; n = 12) or were restricted to 90% (n = 4), 80% (n = 4), or 70% (n = 4) of ad libitum. For the first 3 wk of dietary restriction, food allowances for each of the restricted treatments were calculated using the ad libitum intake of each individual animal recorded during the previous 2 wk. Thereafter, allowances were altered on a weekly basis so that the increase in intake by restrictedly fed animals was always proportional to the increase in intake relative to BW observed in Cal lambs. The design of experiment I and the timing of the procedures are set out in Table 1. The design allowed measurement
of the effects of nematode infection on voluntary ADFI and PLC in lambs fed for ad libitum intake and, by including restrictedly fed lambs, the effect of infection on PLC in lambs with similar intakes as well.

**Experiment II (Secondary Infection)**

**Animals, Housing, and Experimental Design.** Forty-eight of the previously infected lambs, 24 S and 24 B, were used in experiment II. At the beginning of the experiment, all lambs were approximately 6 mo of age. There was a period of 2 wk between the end of the primary infection study and the initiation of a secondary infection, during which all lambs were fed for ad libitum intake. At the end of this period, S and B lambs had mean BW of 50.2 ± 0.75 and 37.6 ± 1.07 kg, respectively. To obtain a good estimate of the effects of reinfection on ADFI and PLC, 12 lambs of each breed were reinfected with the same parasite, dose, and infection regime as described in experiment I and were fed for ad libitum intake. Non-reinfected control lambs received a sham infection at the same time. Eight control lambs in each breed were fed for ad libitum intake. The remaining control lambs in each breed (n = 4) were fed at 90% of ad libitum as described previously. Experiment II lasted for 12 wk, and the experimental facilities and diets and infection details were identical to those used in experiment I. The design of experiment II and the timing of the procedures are set out in Table 1.

**Sample Collection and Measurements**

In both experiments, the measurements and procedures were carried out over similar time scales using identical protocols.

**BW and Food Intake Measurements.** All lambs were weighed once weekly from arrival in the animal house until the end of each experiment. Amounts of distributed food (as fed) were recorded daily, and any accumulated residues were weighed twice weekly (Monday and Thursday). Voluntary ADFI was calculated from the weekly amounts of food offered and weekly refusals.

**Fecal Egg Counts.** For FEC determination, a 5- to 10-g fecal sample was taken from the rectum of all the lambs at weekly intervals from d -10 onwards during the entire experimental period. The individual fecal samples were processed immediately. The number of eggs per gram of fresh feces (epg) was determined using a modified flotation technique (Christie and Jackson, 1982), in which polyallomer centrifuge tubes (Beckman) were used to separate the egg-bearing layer at a rate of 7.5 mg/kg of BW.

**Blood Samples.** Weekly blood samples (approximately 8 mL) were taken from the jugular vein into heparinized Vacutainers (Becton Dickinson, Oxford, UK) from housing until the end of each experiment.

<table>
<thead>
<tr>
<th>Table 1. Summary of experimental design and timing of treatments for experiment I and II</th>
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<tr>
<td><strong>Item</strong></td>
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<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Experiment I (primary infection)</td>
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<tr>
<td>INF</td>
</tr>
<tr>
<td>C_{4x}</td>
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<tr>
<td>C_{50}</td>
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<tr>
<td>C_{50}</td>
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<tr>
<td>C_{70}</td>
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<tr>
<td>Experiment II (secondary infection)</td>
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<tr>
<td>INF2</td>
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<tr>
<td>C_{2,4x}</td>
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<tr>
<td>C_{290}</td>
</tr>
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</table>

1INF = lambs fed ad libitum and infected with 21,000 third-stage larvae (L3) of Teladorsagia circumcincta per week (1 dose of 7,000 L3 3 times/wk for 12 wk; n = 24 per breed); C_{4x} = lambs noninfected fed ad libitum (n = 12 per breed); C_{50} = lambs noninfected and fed at 90% of ad libitum (n = 4 per breed); C_{50} = lambs noninfected and fed at 80% of ad libitum (n = 4 per breed); C_{70} = lambs noninfected and fed at 70% of ad libitum (n = 4 per breed).

2Half of the lambs in each treatment were Suffolk × Greyface and half were Scottish Blackface.

3All lambs were given 10 mL of water orally, 3 times/wk for 12 wk, which did (+) or did not (−) contain 7,000 Teladorsagia circumcincta larvae (i.e., 21,000 larvae/wk). At the end of each infection period (wk 12), the lambs were dewormed with a combination of levamisole at a rate of 0.2 mL/kg of BW and fenbendazole at a rate of 7.5 mg/kg of BW.

4Lambs allocated to the restricted-fed treatments were fed ad libitum for the first 2 wk of the experiment.

5Experiment II (secondary infection) began 2 wk after the end of experiment I. All lambs included in experiment II were allocated from the infected treatment in experiment I.
Blood samples were centrifuged for 15 min at 2,500 × g, and the plasma was separated and then stored at −20°C pending analysis for leptin.

**Leptin RIA**

A ruminant leptin RIA, developed at the Agri-Food and Biosciences Institute, was used to determine PLC. This assay is a double-antibody, ovine-specific RIA developed using purified recombinant ovine leptin (a gift from A. Gertler of The Hebrew University of Jerusalem, Israel). This leptin was used for antibody generation in guinea pigs and also for in-house production of radioiodinated leptin (label).

**Preparation of Radioiodinated Leptin.** Radioiodinated leptin was prepared by iodination with sodium $^{125}$I-iodide (Amersham Pharmacia Biochem, Buckinghamshire, UK) using Iodogen-coated tubes. To an Iodogen-coated tube, 5 μL (500 μCi) of sodium $^{125}$I-iodide solution and 70 μL of 0.1 M Tris-Cl buffer pH 6.8 containing 3 μg of recombinant ovine leptin were added in rapid succession. The contents of the tubes were gently mixed, and the reaction was allowed to proceed for 3 min at room temperature with occasional gentle mixing until termination by addition of 500 μL of PBS with a pH of 6.8. The contents were then immediately transferred to a clean polystyrene tube. After 30 min, the diluted reaction mixture was applied to a precalibrated 25 × 500-mm glass chromatographic column containing Sephadex G-75 (Amersham Pharmacia Biochem) equilibrated in 0.05 M PBS, pH 6.8, containing 0.1% RIA-grade BSA (Sigma Chemical Co., Poole, Dorset, UK). The column was eluted using the same buffer and collecting 2-mL fractions. Aliquots of 5 μL of all fractions were counted for radioactivity in a Cobra II gamma counter (Packard Canberra Ltd., Berkshire, UK), and an overnight binding check (of labeled leptin to antisemur) was made on alternate fractions across the predicted leptin peak. Fractions with satisfactory binding activity were pooled to provide a single stock batch of label that was frozen in appropriately sized aliquots.

**Ovine RIA Protocol and Validation.** The assay standard used in experiment I was pure recombinant ovine leptin (DSL Ltd., London, UK). This was dissolved in 1 mL of deionized water and stored as 20 × 50 μL aliquots (10 ng/μL of stock solution) at −75°C. Stock aliquots were serially diluted with assay buffer to provide standards containing 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.395, 0.1975, and 0.098 ng of leptin/mL. Assays were conducted in polystyrene LP4 tubes (Thermoquest Ltd., Hemel Hempstead, UK) containing 100 μL of sample (in duplicate), 100 μL of assay buffer (0.05 M PBS containing 5 g/L of BSA, 0.5 g/L of Triton X-100, and 0.025 M EDTA disodium salt with addition of 0.2 g/L of sodium azide as a preservative and, if necessary, with the pH adjusted to 7.4 with 0.01 M sodium hydroxide), and 100 μL of antibody (at a final tube dilution of 1:160,000, vol/vol). Standard curve tubes (containing assay standard instead of sample) were prepared in triplicate. Assay tubes were incubated at 4°C for 20 h before addition of 100 μL of $^{125}$I-leptin (14,000 to 16,000 counts/min) in assay buffer. The tubes were incubated for a further 48 h, and then bound and free ligand were separated by adding 100 μL of cellulose-immobilized, anti-guinea-pig IgG (SacCell, IDS, Boldon, Tyne & Wear, UK). After gentle mixing and resting for 30 min at ambient temperature, 1 mL of deionized water was added to all tubes (except total counts tubes). Tubes were centrifuged (1,900 × g; 4°C; 20 min), and the supernatant above each pellet was aspirated by a vacuum pump via a trap. The residual pellets were counted for a minimum of 2 min in a gamma counter. Plasma samples were analyzed in a series of 7 and 2 assays for experiments I and II, respectively, and the assays were balanced internally for breed and treatment. The leptin used for iodination (Gertler ovine leptin) was used as the standard in experiment II.

**Leptin RIA Characteristics.** Because the standards used in the 2 series of assays differed, the leptin results in the 2 experiments are not directly comparable. In experiment I, the assay CV calculated from 6 replicates of an ovine plasma control sample with a mean predetermined leptin concentration of 12.1 ± 0.64 ng/mL was 10.2% (range 5.9 to 14.2%) for the intraassay CV and 13.9% for interassay CV. In experiment II, the mean intra assay CV calculated from 5 replicates of an ovine plasma control sample with a mean predetermined leptin concentration of 5.5 ± 0.23 ng/mL was 12.9% (11.6 and 14.2%). The interassay CV was 13.7%. The sensitivity of the leptin RIA, expressed as the amount of leptin required to reduce by 5% the amount of radiolabelled leptin bound in the absence of competing leptin (Bo) varied between assays and was 0.73 ng/mL or less.

**Statistical Analysis**

Body weight, ADFI, ADFI relative to BW (RADFI, g·d⁻¹·kg⁻¹), and plasma leptin data were analyzed by ANOVA using the MIXED procedure (SAS Inst. Inc., Cary, NC). The statistical model for ADFI, BW, and RADFI contained the fixed effects of breed, infection, sex, time, and interactions, with comparisons based on the data obtained from the ad libitum-fed animals only. Additionally, plasma leptin analysis included the observations of the restrictedly fed animals. Comparison of actual plasma leptin levels across treatments was made by a model that included the main effects of breed, treatment (see Table 1), sex, time, and their interactions (model 1). However, the effect of infection on PLC was assessed through a similar model that contained the RADFI of the lambs as a covariate in addition to the main effects of breed, sex, infection, time, and their interactions (model 2). All models for leptin included an assay effect to take into account the between-assay
variation. In every statistical model, the random effect was animal nested within breed. Sex and its interactions did not affect any of the variables analyzed (in all cases $P > 0.05$) and were therefore ignored in the presentation of results. Data are reported as least squares means and their SEM, and differences were tested by a $t$-test. Body weight gain (kg/wk) for each animal was calculated by linear regression, and the data were analyzed by ANOVA (GLM), with the fixed effects of breed and infection. Effects of breed and infection were analyzed on the basis of data of ad libitum-fed animals only, and data are reported as least squares means and their SEM. Before statistical analysis of FEC, the data were log-transformed according to $\log_{10} (x+1)$, to normalize the residuals. Log-transformed FEC data were analyzed by repeated-measures ANOVA (GenStat Release 7.2, Lawes Agricultural Trust, Rothamsted Experimental Station, Hemel Hempstead, UK). Fecal egg counts data are reported as back-transformed means according to the methods of Johnson et al. (1998) as $10^{(\mu + 0.5 \times \sigma^2)}$, with 95% confidence intervals (CI).

**RESULTS**

**Experiment I (Primary Infection)**

**Fecal Egg Counts.** Fecal samples taken on d −10 from S lambs had 42 epg (95% CI = 31 to 58), and those from B lambs had 18 epg (95% CI = 9 to 35). After the unexpected finding that lambs were not parasite-free, all animals were immediately treated with the anthelmintics fenbendazole and levamisole (same dosage previously mentioned) before being infected according to the experimental protocol. Mean back-transformed FEC for the infected groups are shown in Figure 1a. With the exception of d −10, noninfected lambs (sham-infected) had zero FEC throughout the experiment. In both breeds, FEC reached a maximum value during the third week of infection with 113 epg (95% CI = 81 to 158) for S lambs and 73 epg (95% CI = 51 to 102) for B lambs. From the third week of infection, FEC declined gradually to zero. There was a breed × time interaction ($P = 0.02$), because lambs of the S breed had greater mean FEC than B lambs in the middle, but not at the beginning or the end, of the experimental period (Figure 1a).

**Food Intake.** The mean ADFI for S and B lambs in all treatments are shown in Figures 2a and 2b, respectively. Absolute ADFI was affected by breed ($P < 0.001$; Table 2) and was $2.19 \pm 0.07$ and $1.49 \pm 0.07$ kg/d in $C_{S,E}$ lambs of S and B breed, respectively, but RADFI was not different between breeds ($P = 0.99$; Table 2). However, there was a breed × time interaction for ADFI ($P < 0.001$) and RADFI ($P = 0.01$), indicating that the rate of the increase in ADFI and RADFI was greater in S lambs compared with B lambs (Table 2).

Dosing with parasites caused a significant reduction in ADFI in S lambs, but not in B lambs, as indicated by the interaction between infection and breed ($P = 0.03$; Figure 1a).

**Figure 1.** Mean weekly back-transformed log$_{10}$ fecal egg counts (FEC) in number of eggs per gram of fresh feces (epg) of Suffolk × Greyface (S; ●,○) and Scottish Blackface (B; ■,□) lambs during experiment I (a; primary infection) and experiment II (b; secondary infection). In panel (a), open symbols (○,□) refer to FEC of 24 control S (○) and 24 B (□) lambs measured 1 wk before the beginning of the experiment. In both panels, closed symbols (●,■) refer to FEC of infected S (●) and infected B (■) lambs. During experiment I (n = 24 lambs per breed) and experiment II (n = 12 lambs per breed), infected lambs received orally 21,000 third-stage-larvae (L3) of *Teladorsagia circumcincta* per week (1 dose of 7,000 L3 3 times/wk for 12 wk). Fecal egg counts from the noninfected lambs during both experiments were zero, and are not shown. During experiment I, there was a significant breed effect ($P = 0.007$) and a significant interaction between breed and time ($P = 0.02$). During experiment II, there was no significant effect of breed ($P = 0.9$) or interaction between breed and time ($P = 0.12$).
Figure 2. The (a and b) ADFI, (c and d) BW, and (e and f) plasma leptin concentrations of noninfected lambs fed ad libitum (C_{ad}; —○—), restricted-fed at 90% (C_{90}; —∆—), 80% (C_{80}; —□—), and 70% (C_{70}; —◊—) of ad libitum and infected and fed ad libitum (INF; —●—) Suffolk × Greyface (panels a, c, and e) and Scottish Blackface (panels b, d, and f) lambs during experiment I (primary infection). Infected lambs (n = 24 per breed) received orally 21,000 third-stage-larvae (L3) of Teladorsagia circumcincta per week (1 dose of 7,000 L3 3 times/wk for 12 wk). Noninfected ad libitum-fed lambs (n = 12 per breed) and restricted-fed lambs (n = 12 per breed) received at the same time sham infections. The SEM in each treatment group are shown by vertical bars. The SEM for ad libitum and restricted-fed treatments are based on error mean squares pooled over ad libitum and restricted-fed animals, respectively. The SEM of the leptin data are based on error mean squares pooled over all treatments.
### Table 2. Least squares means of ADFI, ADFI as a proportion of BW (RADFI), BW gain, and plasma leptin concentrations (PLC) of Suffolk × Greyface and Scottish Blackface lambs during experiment I

<table>
<thead>
<tr>
<th>Item</th>
<th>Suffolk × Greyface</th>
<th>Scottish Blackface</th>
<th>SEM²</th>
<th>SEM³</th>
<th>B</th>
<th>I</th>
<th>T</th>
<th>FR</th>
<th>B × I</th>
<th>B × T</th>
<th>RADFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADFI, kg/d</td>
<td>INF 1.93, C₉₀ 2.19, C₈₀ 1.82, C₇₀ 1.53</td>
<td>INF 1.82, C₉₀ 1.53, C₈₀ 1.43, C₇₀ 1.43</td>
<td>0.046 0.666 0.061</td>
<td>&lt;0.001 0.001 &lt;0.001</td>
<td>0.03 0.01 &lt;0.001</td>
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<tr>
<td>RADFI, g d⁻¹ kg⁻¹</td>
<td>INF 49.3, C₉₀ 54.4, C₈₀ 39.8, C₇₀ 39.8</td>
<td>INF 51.8, C₉₀ 52.0, C₈₀ 42.2, C₇₀ 42.3</td>
<td>0.092 0.151 0.132</td>
<td>0.001 0.001 &lt;0.001</td>
<td>0.03 0.01 &lt;0.001</td>
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<tr>
<td>BW gain, kg/wk</td>
<td>INF 1.35, C₉₀ 1.59, C₈₀ 0.97, C₇₀ 0.97</td>
<td>INF 1.20, C₉₀ 1.27, C₈₀ 0.89, C₇₀ 0.75</td>
<td>0.048 0.069 0.11</td>
<td>&lt;0.001 0.015</td>
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<tr>
<td>PLC, ng/mL</td>
<td>INF 5.3, C₉₀ 5.1, C₈₀ 3.9, C₇₀ 3.5</td>
<td>INF 5.0, C₉₀ 4.7, C₈₀ 4.0, C₇₀ 3.6</td>
<td>0.28 0.40 0.69</td>
<td>0.41 0.53 &lt;0.001</td>
<td>0.05 0.07 0.02</td>
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1INF = lambs fed ad libitum and infected with 21,000 third-stage larvae (L₃) of Teladorsagia circumcincta per week (1 dose of 7,000 L₃ 3 times/wk for 12 wk; n = 24 per breed); C₉₀ = lambs noninfected fed ad libitum (n = 12 per breed); C₈₀ = lambs noninfected and fed at 90% of ad libitum (n = 4 per breed); C₇₀ = lambs noninfected and fed at 70% of ad libitum (n = 4 per breed).

2SEM for ad libitum and restricted-fed treatments are based on error mean squares pooled over ad libitum and restricted-fed animals respectively.

3B = breed; I = parasite infection; T = time; FR = food restriction; RADFI = relative ADFI (g d⁻¹ kg⁻¹). The P-values of the effects of B, I, and their interaction were calculated based on error mean squares pooled over ad libitum and restricted-fed animals.

4Calculated based on a statistical model where each treatment was included as a fixed factor. SEM are based on error mean squares pooled over all treatments.

5Calculated based on a model in which RADFI was included as a covariable. NINF = noninfected lambs (n = 12 per breed).

### Table 3. Least squares means of ADFI, ADFI as a proportion of BW (RADFI), BW gain, and plasma leptin concentrations (PLC) of Suffolk × Greyface and Scottish Blackface lambs during experiment II

<table>
<thead>
<tr>
<th>Item</th>
<th>Suffolk × Greyface</th>
<th>Scottish Blackface</th>
<th>SEM²</th>
<th>SEM³</th>
<th>B</th>
<th>I</th>
<th>T</th>
<th>FR</th>
<th>B × I</th>
<th>B × T</th>
<th>RADFI</th>
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<tbody>
<tr>
<td>ADFI, kg/d</td>
<td>INF 2.46, C₉₀ 2.75, C₈₀ 2.27</td>
<td>INF 1.99, C₉₀ 1.99, C₈₀ 1.71</td>
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<tr>
<td>RADFI, g d⁻¹ kg⁻¹</td>
<td>INF 43.8, C₉₀ 45.8, C₈₀ 41.2</td>
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<tr>
<td>BW gain, kg/wk</td>
<td>INF 1.15, C₉₀ 1.46, C₈₀ 1.12</td>
<td>INF 1.19, C₉₀ 0.94, C₈₀ 0.96</td>
<td>0.074 0.084 0.087</td>
<td>0.16 0.07 &lt;0.001</td>
<td>0.42 0.57</td>
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<tr>
<td>PLC, ng/mL</td>
<td>INF 5.7, C₉₀ 4.3, C₈₀ 3.6</td>
<td>INF 4.7, C₉₀ 4.1, C₈₀ 2.6</td>
<td>0.45 0.53 0.78</td>
<td>0.08 0.004 &lt;0.001</td>
<td>0.50 0.22</td>
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1INF = lambs fed ad libitum and infected with 21,000 third-stage larvae (L₃) of Teladorsagia circumcincta per week (1 dose of 7,000 L₃ 3 times/wk for 12 wk; n = 24 per breed); C₉₀ = lambs noninfected fed ad libitum (n = 12 per breed); C₈₀ = lambs noninfected and fed at 90% of ad libitum (n = 4 per breed).

2SEM for ad libitum and restricted-fed treatments are based on error mean squares pooled over ad libitum and restricted-fed animals respectively.

3B = breed; I = parasite infection; T = time; FR = food restriction; RADFI = relative ADFI (g d⁻¹ kg⁻¹). The P-values of the effects of B, I, and their interaction were calculated based on error mean squares pooled over ad libitum and restricted-fed animals.

4Calculated based on a statistical model where each treatment was included as a fixed factor. SEM are based on error mean squares pooled over all treatments.

5Calculated based on a model in which RADFI was included as a covariable. NINF = noninfected lambs (n = 12 per breed).
Table 2). The difference in ADFI between INF and C_{all} S lambs was around 12% and was persistent throughout the course of the 12-wk parasitic challenge (Figure 2a). Similarly, RADFI was decreased by infection in S lambs, but not in B lambs, as indicated by the interaction between infection and breed (P = 0.03; Table 2).

**BW and BW Gain.** The average weekly BW for S and B lambs is shown in Figures 2c and 2d, respectively. Ad libitum-fed lambs of the B breed had approximately 16% lower BW gain compared with S lambs (P < 0.001; Table 2). Infection affected the BW gain of lambs (P = 0.01; Table 2), with a reduction in BW gain of 15 and 7% in S and B lambs, respectively, but the interaction between breed and infection was not significant (P = 0.15, Table 2). All levels of food restriction resulted in lower BW gain in both breeds (P < 0.001; Table 2).

**PLC.** Figures 2e and 2f show the mean PLC during experiment I of S and B lambs, respectively. A direct comparison of actual PLC across treatments, without taking into account the RADFI of the lambs in each treatment (model 1), indicated no differences in PLC between INF and C_{all} lambs in both breeds (P = 0.53; Table 2). Restrictedly fed lambs had lower PLC compared with the ad libitum-fed lambs in both breeds (P < 0.05; Table 2). However, when the highly significant effect of covariable RADFI on PLC was taken into account (model 2), there was a significant positive effect of infection (P = 0.02; Table 2) on PLC. Both models showed that PLC was not affected by breed and increased with time (P < 0.001; Table 2).

**Experiment II (Secondary Infection)**

**Fecal Egg Counts.** Mean weekly FEC remained very low and did not differ between breeds (P = 0.9; Figure 1b).

**Food Intake.** The mean ADFI for S and B lambs in all treatments are shown in Figures 3a and 3b, respectively. Absolute ADFI was affected by breed with 2.75 ± 0.087 and 1.99 ± 0.087 kg/d for the C_{all} lambs of S and B breed, respectively (P < 0.001; Table 3). During the course of the secondary parasitic infection, the pattern of ADFI of INF and C_{all} lambs in the 2 breeds was similar to that observed during the primary infection. The interaction between breed and infection tended to be significant (P = 0.08; Table 3), and this was due to the difference of around 12% in ADFI between INF and C_{all} S lambs but not B lambs (Figures 3a and 3b).

**BW and BW Gain.** The average weekly BW in S and B lambs is shown in Figures 3c and 3d, respectively. The 2 breeds differed in BW gain with S lambs gaining 36% more BW than B lambs (P = 0.005; Table 3). Secondary infection with *T. circumcincta* resulted in a significant reduction in BW gain in S lambs, but not in B lambs, as indicated by the significant interaction between breed and infection (P = 0.001; Table 3).

**PLC.** Figures 3e and 3f show the mean PLC in S and B lambs during experiment II. The results of the analysis of model 1 showed no differences in PLC between restrictedly fed and C_{all} lambs (Table 3). Plasma leptin concentrations tended to be greater in INF lambs than in C_{all} lambs (P = 0.07; Table 3). However, the effect of covariable RADFI on PLC was highly significant, and when this was taken into account (model 2), infection had a positive effect on PLC (P = 0.004; Table 3). Both models showed that PLC was not affected by breed and increased with time (P < 0.001; Table 3).

**DISCUSSION**

The findings of this study relate to the differences in response to infection between the lambs of the 2 breeds, the effects of infection on ADFI, and the effect of infection on the relationship between PLC and ADFI in lambs.

**Breed Effects**

The breeds used in the present study are known to vary in their intrinsic capacity for growth and mature size in a common environment (Emmans and Friggens, 1995; Lewis et al., 2004). The performance of the ad libitum-fed S lambs was indeed superior to that of the ad libitum-fed B lambs in both experiments I (P < 0.001) and II (P = 0.005), as expected.

Previous studies have reported that sheep selected more intensively for high productivity, such as fast growth or high fleece weight, are more susceptible to parasitic infection, as evidenced by high FEC and worm burdens, than animals selected less intensively (McEwan et al., 1992; Miller et al., 1998; Amarante et al., 2004). According to a recently developed framework, susceptible animals can be expected to show a larger depression in intake that lasts longer than the anorexia in less susceptible animals (Sandberg et al., 2006). Our study is the first to investigate the effect of breeds of different production potential on the degree of anorexia in sheep. The first aim of our study was to test the hypothesis that variation in response to infection as measured by FEC would be associated with variation in the degree of anorexia after parasitic infection. In addition, our aim was to test whether such an infection would result in elevated PLC and whether breed differences in anorexia would be positively correlated with breed differences in PLC.

The results obtained are consistent with these expectations and seem, at least at first sight, to support the hypotheses. Primary parasite infection resulted in a greater FEC in S than in B lambs. In both breeds, animals began to show moderate levels of eggs in their feces by the second week of dosing and rose to a maximum value during the third week of infection (Figure 1a). From the fourth week of infection and onwards, FEC gradually declined to zero, which suggests that development of immunity had begun to occur. This pattern of FEC is consistent with experimental infections with *T. circumcincta* (Coop et al., 1977, 1982), in which the greatest value is observed within 3 to 4 wk postin-
Figure 3. The (a and b) ADFI, (c and d) BW, and (e and f) plasma leptin concentrations of noninfected fed ad libitum (C<sub>ad</sub>; —○—), restricted-fed at 90% (C<sub>90</sub>; —△—) of ad libitum, and infected and fed ad libitum (INF; —●—) Suffolk × Greyface (panels a, c, and e) and Scottish Blackface (panels b, d, and f) lambs during experiment II (secondary infection). Infected lambs (n = 12 per breed) received orally 21,000 third-stage-larvae (L<sub>3</sub>) of *Teladorsagia circumcincta* per week (1 dose of 7,000 L<sub>3</sub> 3 times/wk for 12 wk). Noninfected ad libitum-fed lambs (n = 8 per breed) and restricted-fed lambs (n = 4 per breed) received at the same time sham infections. The SEM in each treatment are shown by vertical bars. The SEM for ad libitum and restricted-fed treatments are based on error mean squares pooled over ad libitum and restricted-fed animals, respectively. The SEM of the leptin data are based on error mean squares pooled over all treatments.
fection. The absolute FEC values were relatively low compared with previous studies with similar doses of infection in parasite-naive lambs (Symons et al., 1981; Coop et al., 1982), but infection level was sufficient to affect ADFI. Although precautions had been taken to avoid exposure to parasites, FEC measured at housing, although low, were not zero (Figure 1a). Hence, lambs were not entirely parasite-naive before the beginning of the experiment. Because the pasture was newly established and had never been grazed before, it seems likely that the anthelmintic treatment applied to the ewes had not been completely effective. Consequently, it is likely that the worm eggs excreted by the ewes were the source of infection to their lambs, resulting in the low FEC observed 10 d before the beginning of the primary infection. During the course of the secondary infection, FEC remained very low, and no differences were noted between the breeds (Figure 1b). Because there is evidence that animals can lose some of their acquired immunity when their exposure to pathogens is discontinued (Barger, 1988; Jackson et al., 2004), these findings suggest that a period of 2 wk between a primary and a secondary infection will not result in a significant loss of immunity in these breeds.

There were significant breed effects on anorexia, as hypothesized. Infected B lambs did not differ in ADFI from noninfected lambs during either primary or secondary infection. This means that, with the exception of a very small increase in FEC during primary infection, B lambs did not respond to infection at all. This virtual absence of effects of infection on B lambs was unexpected. Much more pronounced responses, in terms of FEC and ADFI, to infection using the same model have been amply demonstrated in many genotypes of lambs (Coop et al., 1977; Sykes and Coop, 1977; Symons et al., 1981). In their review, Coop and Holmes (1996) characterized Scottish Blackface as a relatively parasite-resistant breed based on FEC data obtained with *Heteropogon contortus* infection. However, to our knowledge, effects of infection with *T. circumcincta* on ADFI of B lambs have not been investigated before, and, therefore, no comparisons with our data are available.

The low FEC and the absence of anorexia in B lambs could have been related to food quality. As judged from animal growth rates, the quality of the food allowed for good performance. Fecal egg counts are generally greater in lambs with access to poor rather than high quality food (Van Houtert and Sykes, 1996). According to a nutrient partitioning framework developed by Coop and Kyriazakis (1999), nutritional limitation can affect lambs of greater production potential more than animals of lower production potential. Greater growth rates of B and S crossbreds fed high-quality food have been observed in our institute before (Lewis et al., 2004), suggesting that the nutrient content of the food used in our experiments was limiting lamb performance, and this may have affected the 2 genotypes differently. In addition, lambs were not completely parasite-naive before the experiment began, as discussed earlier, and previous exposure to parasites could have affected the response of B lambs differently from S lambs in the first experiment. At the beginning of experiment II, however, lambs of both breeds had been exposed to *T. circumcincta* for 12 wk. Differences in previous exposure cannot, then, explain the breed effects on anorexia. We can conclude, therefore, that in terms of FEC and anorexia, S lambs were affected more by nematode infections than B lambs when both breeds had access to the food provided in our experiments.

**Effects of (Re)infection on Food Intake**

During the course of primary infection, a significant reduction in ADFI (anorexia) of about 13% was observed from the second week of infection in parasitized S lambs. This depression of intake was persistent throughout the course of the 12-wk parasitic challenge (Figure 2a), which is a common observation in crossbred lambs continuously infected with *T. circumcincta* (Coop et al., 1977; Sykes and Coop, 1977; Symons et al., 1981). This long-lasting depression of appetite is very likely to be directly related to the immune response of the host to gastrointestinal parasites (Greer et al., 2005) rather than the presence of parasites per se (Coop et al., 1977; Sykes and Coop, 1977).

Ruminants are frequently exposed to infective forms of nematode parasites, but there is very little information on whether reexposure of previously infected animals results in renewed reduction in ADFI. A recent study has shown that reinfection did not result in anorexia in previously infected nonpregnant or nonlactating ewes (Greer et al., 2005). However, the present study showed that reinfection with *T. circumcincta* resulted in a reduction in ADFI of S lambs that was similar to the reduction observed during primary infection in the same breed (around 12%). The interaction between breed and infection was significant during primary infection but only tended to be significant during reinfection, which is likely related to the fewer number of lambs included in experiment II. This suggests that reexposure to nematode infection can cause anorexia in lambs, as has been observed previously in other species (Mercer et al., 2000; Houdijk et al., 2003). In the current study, anthelmintic treatment was followed by a parasite-free period of 2 wk before reinfection began, which is a similar design to that reported in a ewe study (Greer et al., 2005). The difference in findings with ewes and lambs are, therefore, not the result of differences in the length of the parasite-free period. Because young animals have been observed to be more susceptible to infection than adult sheep, the reoccurrence of anorexia in lambs, but not in ewes, may be a result of the difference in age and thus in susceptibility to infection (Smith et al., 1985).

An alternative interpretation of our data is that deworming resulted in a recovery of ADFI in the control animals, whereas reexposure to parasites prevented recovery in the reinfection treatment. This view is
consistent with the finding that frequent anthelmintic treatment did not remove the depressant effect of a continuing *T. circumcincta* infection on voluntary ADFI of lambs (Coop et al., 1982). Although the RADFI of infected lambs during reinfection was not significantly lower than that of control animals, this is a common phenomenon observed in parasitized lambs as discussed by Sykes (1982). However, effects of reinfection after longer parasite-free periods on subsequent ADFI of sheep remain to be investigated.

**Effects of (Re)infection on PLC in Relation to Food Intake**

The relationship between PLC and ADFI during infection is complicated. Previous studies show that a reduction in ADFI as a result of restricted feeding in healthy animals, including sheep, results in a reduction in PLC (Blache et al., 2000; Delavaud et al., 2000; Marie et al., 2001; Morrison et al., 2001). Nematode infection was, therefore, expected to be associated with a decrease in PLC. Nevertheless, the literature shows that infection and the subsequent immune response are associated with an increase in PLC in several infectious diseases, including parasitism, in murine animal models (Grunfeld et al., 1996; Barbier et al., 1998; Fantuzzi and Faggioni, 2000; Mercer et al., 2000; Faggioni et al., 2001). As a result, PLC in infected animals showing anorexia is likely to result from both a stimulating effect (by infection) and a suppressive effect (from the reduction in ADFI). Such opposing effects on PLC have been suggested for endotoxemia in pigs (Barb et al., 1998), and it has been proposed that the same effects could be relevant during infections in ruminants (Kulcsar et al., 2005). In the current study, we tested the hypothesis that nematode infection will result in elevated PLC in lambs with similar ADFI to noninfected lambs. For that reason, restrictedly fed control lambs were included in the experiment to be able to quantify effects of ADFI on PLC in the absence of infection. The reduction in ADFI of restrictedly fed control lambs of both breeds resulted in systematic decreases in PLC compared with ad libitum-fed control lambs in both experiments (Figures 2e, 2f, and 3e, 3f), which is consistent with previous observations (Marie et al., 2001; Morrison et al., 2001; Delavaud et al., 2002).

The effects of nematode infection on PLC in ruminants have not been studied extensively. Data obtained in a recent study (Fox et al., 2006) suggested that infection with *T. circumcincta* results in elevation of PLC during d 5 to 13 after infection. However, these results are difficult to interpret because of the absence of any noninfected control lambs in that experiment. In contrast, our study allowed a direct comparison of PLC between infected and noninfected ad libitum-fed lambs. Our results show that during the course of the primary parasitic infection, actual PLC, as analyzed by model 1, did not differ significantly between infected and noninfected ad libitum-fed lambs. Although PLC tended to be greater in infected lambs, especially in the S breed (Figure 3e) during reinfection, the effect was not statistically significant. These findings are in contrast to the acute increase in PLC that has been observed in earlier studies during nematode infection (Roberts et al., 1999; Mercer et al., 2000), acute intestinal inflammation (Barbier et al., 1998), and other disease models in rodents (Grunfeld et al., 1996; Faggioni et al., 1997; Sarraf et al., 1997). They are, however, consistent with the lack of effect on PLC of acute endotoxemia in sheep (Soliman et al., 2001) and cows (Soliman et al., 2002) and *Salmonella* infections in pigs (Jenkins et al., 2004). Gastrointestinal nematode infection does, therefore, not seem to result in an acute increase of PLC in ad libitum-fed sheep.

In restrictedly fed sheep, infection with a combination of *T. circumcincta* and *Trichostrongylus colubriformis* did not result in an increase in PLC (Liu et al., 2007). The present study is the first to allow a comparison of PLC of ad libitum-fed infected with noninfected lambs with similar levels of RADFI. Analysis of our data with model 2, where RADFI was included as a covariable, showed that during primary infection, infected lambs had greater PLC than noninfected lambs with similar RADFI. This effect of infection on PLC was also observed during secondary infection (Table 3). These results are in agreement with the first hypothesis we set out to test (i.e., that nematode infection can have a positive effect on plasma leptin levels in lambs when the effect of infection on ADFI is taken into account). Therefore, the current study provides some evidence for the suggestion of Kulcsar et al. (2005) that 2 opposing mechanisms (stimulation by inflammation and inhibition by reduced ADFI-energy metabolism) are likely to affect PLC during infection in sheep. It is, therefore, possible that leptin is involved in the immune response to nematode infection in lambs. Although such a role for leptin has also been suggested in another recent study with parasitized periparturient ewes (Valderrabano et al., 2006), the importance of this role in sheep remains to be established.

The literature also shows that in ad libitum-fed healthy animals, an increase in plasma leptin level is associated with a decrease in voluntary ADFI, and leptin administration promotes anorexia (Henry et al., 1999; Morrison et al., 2001). If an increase in plasma leptin level depresses ADFI in lambs, this mechanism could play an important role in the infection-related anorexia that is observed in many disease models. Indeed, such a role for leptin has been proposed in several species (Grunfeld et al., 1996; Sarraf et al., 1997; Mercer et al., 2000). However, other studies have suggested that leptin itself is not responsible alone for the occurrence of anorexia during infection (Faggioni et al., 1997). For example, the parasite-induced anorexia in rats was suggested to be related to the cytokine interleukin 6, which is released during the immune response and is a known mediator of anorexia in the acute-phase immune response, rather than to the elevated leptin
concentrations observed during early infection (Roberts et al., 1999). In our study, parasitic infection did not result in an acute increase in PLC during both the primary and the secondary infection. In addition, although infected lambs had significantly greater PLC than noninfected lambs with similar ADFI in both breeds, anorexia was observed in lambs of the S breed only. These effects were observed during both the primary and the secondary infection. Therefore, these results show that, although leptin may be involved in the response of lambs to nematode infection, it is highly unlikely that leptin alone is responsible for the anorexia that is observed in parasitized lambs.

In conclusion, the data show that there were differences between B and S lambs in their response to nematode infection with *T. circumcincta*. The S lambs were more susceptible to infection than B lambs as evidenced by the larger increase in FEC, and S lambs also developed anorexia whereas B lambs did not. The data are, therefore, the first to show that infection with *T. circumcincta* depresses the ADFI of lambs of a susceptible breed more than that of lambs of a less susceptible breed to parasitic infection. In addition, the study demonstrated that susceptible lambs that are re-exposed to infection can show anorexia again. During both primary and secondary infection, infected lambs had greater PLC than noninfected lambs with similar ADFI. The results show that leptin may be involved in the response of lambs to infection but that it is unlikely that leptin alone is responsible for the anorexia that is observed after parasitic infection.

**LITERATURE CITED**


